

## Supporting Information – Text S2

### Derivation of the equations presented in the paper

#### Calculation of the equilibrium concentration of all chemical species

The following equilibrium constants are defined based on the thermodynamic cycle shown in Scheme 1:

$$K_{OpH} = \frac{(Op^-)(H^+)}{(OpH)} \quad (1)$$

$$K_{CH} = \frac{(C^-)(H^+)}{(CH)} \quad (2)$$

$$K_H = \frac{(OpH)}{(CH)} \quad (3)$$

$$K_D = \frac{(Op^-)}{(C^-)} \quad (4)$$

Using mole fractions, the following must also be true:

$$(Op^-) + (OpH) + (C^-) + (CH) = 1 \quad (5)$$

From the thermodynamic cycle,

$$-RT \ln K_{OpH} = RT \ln K_H - RT \ln K_{CH} - RT \ln K_D \quad (6)$$

Rearranging this equation, it is possible to obtain an expression for  $K_H$  as a function of  $K_D$ :

$$K_H = \frac{K_{CH} \times K_D}{K_{OpH}} \quad (7)$$

By replacing  $K_H$  from equation (7) into equation (3), we get:

$$(OpH) = \frac{(CH) \times K_{CH} \times K_D}{K_{OpH}} \quad (8)$$

Using (8), (4) and (2) into (5), it is possible to obtain an equation for  $C^-$  as a function of  $K_D$ ,  $(H^+)$ ,  $K_{OpH}$ ,  $K_{CH}$ :

$$(C^-) = \frac{1}{1 + K_D + (H^+) \left( \frac{K_D}{K_{OpH}} + \frac{1}{K_{CH}} \right)} \quad (9)$$

Then, the mole fraction of all NP4 states can be calculated using equation (9), together with (1), (2) and (4), for a given solvent pH and  $K_D$  value:

$$(CH) = \frac{(H^+)}{K_{CH} \left[ 1 + K_D + (H^+) \left( \frac{K_D}{K_{OpH}} + \frac{1}{K_{CH}} \right) \right]} \quad (10)$$

$$(\text{Op}^-) = \frac{K_D}{1+K_D+(H^+) \left( \frac{K_D}{K_{\text{OpH}}} + \frac{1}{K_{\text{CH}}} \right)} \quad (11)$$

$$(\text{OpH}) = \frac{K_D}{K_{\text{OpH}} \left[ 1+K_D+(H^+) \left( \frac{K_D}{K_{\text{OpH}}} + \frac{1}{K_{\text{CH}}} \right) \right]} \quad (12)$$

### Calculation of the apparent $pK_a$

The experimental signal was defined in equation 2 of the paper as

$$S = \alpha([C^-] + [CH]) + \beta([\text{Op}^-]+[\text{OpH}]) \quad (13)$$

The apparent  $pK_a$  is then defined as the inflection point of the S vs. pH curve and can be calculated as the pH for which the second derivative of S equals zero. This calculation was performed using Mathematica (Wolfram Research, Inc., Mathematica, Version 7.0, Champaign, IL (2008)). The obtained apparent  $pK_a$  is independent of the constants  $\alpha$  and  $\beta$ :

$$\text{apparent } pK_a = pK_{\text{OpH}} + pK_{\text{CH}} - \log((K_D+1)/(K_D \times K_{\text{CH}}+K_{\text{OpH}})) \quad (14)$$